

**TITLE OF INVENTION****DNA IMMUNIZATION AGAINST *CHLAMYDIA* INFECTION****FIELD OF INVENTION**

The present invention relates to immunology and, in particular, to  
5 immunization of hosts using nucleic acid to provide protection against infection by  
*Chlamydia*.

**BACKGROUND OF THE INVENTION**

DNA immunization is an approach for generating protective immunity  
against infectious diseases (ref. 1 - throughout this application, various references  
10 are cited in parentheses to describe more fully the state of the art to which this  
invention pertains. Full bibliographic information for each citation is found at the  
end of the specification, immediately preceding the claims. The disclosure of these  
references are hereby incorporated by reference into the present disclosure). Unlike  
protein or peptide based subunit vaccines, DNA immunization provides protective  
15 immunity through expression of foreign proteins by host cells, thus allowing the  
presentation of antigen to the immune system in a manner more analogous to that  
which occurs during infection with viruses or intracellular pathogens (ref. 2).  
Although considerable interest has been generated by this technique, successful  
immunity has been most consistently induced by DNA immunization for viral  
20 diseases (ref. 3). Results have been more variable with non-viral pathogens which  
may reflect differences in the nature of the pathogens, in the immunizing antigens  
chosen, and in the routes of immunization (ref. 4). Further development of DNA  
vaccination will depend on elucidating the underlying immunological mechanisms  
and broadening its application to other infectious diseases for which existing  
25 strategies of vaccine development have failed.

*Chlamydia trachomatis* is an obligate intracellular bacterial pathogen which  
usually remains localized to mucosal epithelial surfaces of the human host.  
Chlamydiae are dimorphic bacteria with an extracellular spore-like transmission cell  
termed the elementary body (EB) and an intracellular replicative cell termed the  
30 reticulate body (ref. 5). From a public health perspective, chlamydial infections are  
of great importance because they are significant causes of infertility, blindness and

are a prevalent co-factor facilitating the transmission of human immunodeficiency virus type 1 (ref. 6). Protective immunity to *C. trachomatis* is effected through cytokines released by Th1-like CD 4 lymphocyte responses and by local antibody in mucosal secretions and is believed to be primarily directed to the major outer  
5 membrane protein (MOMP), which is quantitatively the dominant surface protein on the chlamydial bacterial cell and has a molecular mass of about 40 kDa (ref. 16).

Initial efforts in developing a chlamydial vaccine were based on parenteral immunization with the whole bacterial cell. Although this approach met with success in human trials, it was limited because protection was short-lived, partial  
10 and vaccination may exacerbate disease during subsequent infection episodes possibly due to pathological reactions to certain chlamydial antigens (ref. 8). More recent attempts at chlamydial vaccine design have been based on a subunit design using MOMP protein or peptides. These subunit vaccines have also generally failed, perhaps because the immunogens do not induce protective cellular and  
15 humoral immune responses recalled by native epitopes on the organism (ref. 9).

In copending US Patent Application No. 08/893,381 filed July 11, 1997, assigned to University of Manitoba and the disclosure of which is incorporated herein by reference (WO 98/02546), I have described the generation of a protective immune response using a DNA sequence which encodes the MOMP of *C.*  
20 *trachomatis* in a plasmid by DNA immunization.

#### SUMMARY OF THE INVENTION

The present invention is concerned with nucleic acid immunization, specifically DNA immunization, to generate in a host protective antibodies to a serine-threonine kinase of a strain of *Chlamydia*. DNA immunization induces a  
25 broad spectrum of immune responses including Th1-like CD4 responses and mucosal immunity.

Accordingly, in one aspect, the present invention provides a non-replicating vector comprising a nucleotide sequence encoding a serine-threonine kinase (STK) or a fragment of STK that generates a STK-specific immune response, and a  
30 promoter sequence operatively coupled to said nucleotide sequence for expression of said STK in a host to which the vector is administered.

The promoter may be a cytomegalovirus promoter, and may be contained in the human cytomegalovirus major immediate-early promoter-enhancer region. The vector may be a plasmid vector and the nucleotide sequence may be that of SEQ ID No: 1.

5           The strain of *Chlamydia* may be a strain of *Chlamydia* inducing chlamydial infection of the lung, including *Chlamydia trachomatis* or *Chlamydia pneumoniae*. The non-replicating vector may be plasmid pcDNA3 into which the nucleotide sequence is inserted. The pcDNA3 vector may contain the nucleotide sequence having SEQ ID No: 1.

10           In a further aspect of the present invention, there is provided an immunogenic composition for *in vivo* administration to a host for the generation in the host of a protective immune response to a serine-threonine kinase (STK) of a strain of *Chlamydia*, comprising a non-replicating vector as provided herein and a pharmaceutically-acceptable carrier therefor.

15           In an additional aspect of the invention, there is provided a method of immunizing a host against disease caused by infection with a strain of *Chlamydia*, which comprises administering to said host an effective amount of a non-replicating vector as provided herein.

20           In these aspects of the present invention, the various options and alternatives discussed above for the non-replicating vector may be employed.

The non-replicating vector may be administered to the host, including a human host, in any convenient manner, such as intramuscularly or intranasally.

25           The present invention also includes, in a further aspect thereof, a method of using a gene encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* or a fragment of said STK that generates a STK-specific immune response, to produce an immune response in a host, which comprises isolating said gene; operatively linking said gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of said STK or fragment thereof when introduced into a host to produce an immune response to said STK or fragment thereof; and introducing said vector into a host.

30

In an additional aspect of the invention, there is provided a method of producing a vaccine for protection of a host against disease caused by infection with a strain of *Chlamydia*, which comprises isolating a nucleotide sequence encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* or a fragment of the STK that generates a STK-specific immune response, operatively linking said nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of said STK or fragment thereof when introduced to a host to produce an immune response to said STK or fragment thereof, and formulating said vector as a vaccine for *in vivo* administration to a host.

The various options and alternatives discussed above may be employed in this aspect of the invention.

Advantages of the present invention, therefore, include a method of obtaining a protective immune response to infection carried by a strain of *Chlamydia* by DNA immunization of DNA encoding the major outer membrane protein of a strain of *Chlamydia*.

#### **BRIEF DESCRIPTION OF DRAWINGS**

Figure 1A and Figure 1B show the results of immunization with serine-threonine kinase gene (pSTK) resulting in enhanced clearance of mouse pneumonitis (MoPn) infection in lung. Groups Balb/c mice were immunized with pSTK (n=5), pcDNA3 (n=6), saline (n=5) or with 1000 IFU of live MoPn EB (n=6). Fourteen days after last immunization, mice were challenged intranasally with infectious MoPn (2000 IFU). Figure 1A: body weight of the mice was measured daily after challenge infection until mice were sacrificed at day 10. Figure 1B: mice were sacrificed at postinfection day 10, and MoPn growth in lung was analyzed by quantitative tissue culture. Data are mean  $\pm$  SE of log<sub>10</sub> IFU/lung. \*p<0.05, p<0.01 vs. pcDNA-treated group. Legend: EB=host-killed elementary bodies, STK=plasmid DNA, N=native, pcDNA3=empty vector.

Figure 2 shows the construction of plasmid pcDNA3/STK.

Figure 3 shows the nucleic acid sequence of the STK gene (SEQ ID No: 1).

### GENERAL DESCRIPTION OF THE INVENTION

To illustrate the present invention, plasmid DNA was constructed containing the serine-threonine kinase (STK) gene from the *C. trachomatis* mouse pneumonitis strain (MoPn), which is a natural murine pathogen, permitting experimentation to be effected in mice. It is known that primary infection in the mouse model induces strong protective immunity to reinfection. For human immunization, a human pathogen strain is used.

Any convenient plasmid vector may be used, such as pcDNA3, a eukaryotic II-selectable expression vector (Invitrogen, San Diego, CA, USA), containing a human cytomegalovirus major-immediate-early promoter-enhancer region. The STK gene may be inserted in the vector in any convenient manner. The gene may be amplified from *Chlamydia trachomatis* genomic DNA by PCR using suitable primers and the PCR product cloned into the vector. The STK gene-carrying plasmid may be transferred, such as by electroporation, into *E. coli* for replication therein. Plasmids may be extracted from the *E. coli* in any convenient manner.

The plasmid containing the STK gene may be administered in any convenient manner to the host, such as intramuscularly or intranasally, in conjunction with a pharmaceutically-acceptable carrier.

The data presented herein and described in detail below demonstrates that DNA immunization with the *C. trachomatis* STK gene elicits immune responses and produces significant protective immunity to lung challenge infection with *C. trachomatis* MoPn.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of chlamydial infections. A further non-limiting discussion of such uses is further presented below.

#### **1. Vaccine Preparation and Use**

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the STK genes and vectors as disclosed herein. The vaccine elicits an immune response in a subject which includes the production of anti-STK antibodies. Immunogenic compositions, including vaccines, containing the nucleic acid may be

prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 9324640) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactide-co-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain an absorption enhancer.

The STK gene containing non-replicating vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary  
5 substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic compositions formed  
10 according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intra-gastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and  
15 carriers may include, for example, polyalkylene glycols or triglycerides. Oral formulations may include normally employed excipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be  
20 therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the STK and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgement  
25 of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1  $\mu$ g to about 1 mg of the STK gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of  
30 administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which

contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

5 Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect  
10 facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have  
15 been identified that enhance the immune response to antigens. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines.

20 A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens to produce immune stimulating complexes (ISCOMS), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP)  
25 and lipopolysaccharide (LPS), as well as monophoryl lipid A, QS 21 and polyphosphazene.

In particular embodiments of the present invention, the non-replicating vector comprising a first nucleotide sequence encoding a STK gene of *Chlamydia* may be delivered in conjunction with a targeting molecule to target the vector to  
30 selected cells including cells of the immune system.



The non-replicating vector may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 14) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth  
5 et al. (ref. 15) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

## 2. Immunoassays

The STK genes and vectors of the present invention are useful as immunogens for the generation of anti-STK antibodies for use in immunoassays,  
10 including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the STK. These STK specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies, such as  
15 the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antibodies, a nonspecific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the  
20 background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with  
25 diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20 to 37 C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific  
30 immunocomplexes between the test sample and the bound STK specific antibodies,

and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

### EXAMPLES

The above disclosure generally describes the present invention. A more  
5 complete understanding can be obtained by reference to the following specific  
Examples. These Examples are described solely for purposes of illustration and are  
not intended to limit the scope of the invention. Changes in form and substitution of  
equivalents are contemplated as circumstances may suggest or render expedient.  
Although specific terms have been employed herein, such terms are intended in a  
10 descriptive sense and not for purposes of limitation.

#### Example 1:

This Example shows the preparation of a plasmid vector for immunization.

The *C. trachomatis* mouse pneumonitis (MoPn) isolate was grown in HeLa  
229 cells in Eagle MEM containing 10% fetal bovine serum and 2 mM L-glutamine.  
15 The MoPn EBs were harvested and purified by step gradient density centrifugation  
at 43,000g for 60 min at 4°C. The purified EBs were washed twice with PBS,  
centrifuged at 30,000g for 30 min, resuspended in sucrose-phosphate-glutamic acid  
(SPG) buffer and frozen at -70°C until used.

The serine-threonine kinase (STK) gene was cloned into eukaryotic  
20 expression plasmid, pcDNA3 (Invitrogen, San Diego) to form plasmid  
pcDNA3/STK. The STK gene was amplified from MoPn genomic DNA by  
polymerase chain reaction (PCR) with a 5' primer (GGG GAT CCG CCA CCA  
TGC TTG AAT TAG GCG TAT CGT TTC CT - SEQ ID No: 2) which included a  
*Bam*HI site, a start codon, and the N-terminal sequence of the mature serine-  
25 threonine kinase of MoPn and a 3' primer (GGG GCT CGA GCT ATT ACC GGA  
CTC TTT TTA AGC TGA TAA G - SEQ ID No: 3) which include a *Xho*I site, two  
stop codons (CTA TTA), and the C-terminal sequence of the *MoPn* STK gene. After  
digestion with *Bam*HI and *Xho*I, the PCR product, having the sequence shown in  
Figure 3 (SEQ ID No: 1), was cloned into *Bam*HI and *Xho*I restricted pcDNA3 with  
30 transcription under the control of human cytomegalovirus major intermediate-early  
promoter-enhancer region. The STK gene-encoding plasmid was transferred by

electroporation into *Escherichia coli* DH5 $\alpha$ , which was grown in Luria-Bertani (LB) broth containing 100  $\mu$ g/ml ampicillin. The plasmid was extracted by a DNA purification system (Wizard Plus Maxiprep; Promega, Madison, WI), and the sequence of recombinant STK DNA was verified by PCR direct sequence analysis.

- 5 Purified plasmid DNA was dissolved in saline at a concentration of 1 mg/ml. The DNA concentration was determined by spectrophotometry (DU-62; Beckman, Fullerton, CA) at 260 nm, and the size of the plasmid was compared with DNA standards in a ethidium bromide-stained agarose gel.

Example 2:

- 10 This Example shows the results of immunizing studies using the plasmid vector.

Female Balb/c mice (4 to 5 weeks old) were purchased from Charles River Canada (St. Constant, Canada) mice were intramuscularly and intranasally immunized with plasmid DNA, prepared as described in Example 1, on three  
15 occasions, at 0, 2 and 4 weeks. For each immunization, a total of 200  $\mu$ g DNA in 200  $\mu$ l was injected into the two quadriceps muscles (100  $\mu$ g of DNA/injection site) using a 27-gauge needle. At the same time, 50  $\mu$ g DNA in 50  $\mu$ l was delivered onto the nostrils of mice with a micropipette. The droplet was subsequently inhaled by the mice.

- 20 Mice were challenged intranasally with  $2 \times 10^3$  IFU of *C. trachomatis* MoPn EB 14 days after last immunization, as described. Briefly, after ether anesthesia 25  $\mu$ l of SPG containing an inoculum of  $2 \times 10^3$  IFU of MoPn was delivered onto the nostrils of mice with a micropipette. The droplet was subsequently inhaled by the mice. Body weight was measured daily for 10 days following the challenge infection  
25 as a measure of chlamydia-induced morbidity. On postinfection day 10, the mice were sacrificed and their lungs were aseptically isolated and homogenized with grinder in SPG buffer. The tissue suspensions were centrifuged at 500g for 10 min at 4°C remove coarse tissue and debris. Supernatants were frozen at -70°C until tissue culture testing for quantitative growth of the organism.

- 30 For more direct measure of the effectiveness of the DNA vaccination, the ability to limit the *in vivo* growth of *Chlamydia* following a sublethal lung infection

was evaluated. In this infection model system, postchallenge day 10 is the time of peak growth and was chosen for comparison of lung titers among the various groups of mice. Mice immunized with STK DNA had a lung titer ( $\log_{10}$  IFU) is 31.6 and 316.2 folds lower than negative control groups (blank vector and saline groups).

5

#### SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by strain of *Chlamydia*, specifically *C. trachomatis*, employing a non-replicating vector, specifically a plasmid vector, containing a  
10 nucleotide sequence encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* and a promoter to effect expression of STK in the host. Modifications are possible within the scope of this invention.

**REFERENCES**

1. M.A. Liu, M.R. Hilleman, R. Kurth, Ann. N.Y. Acad. Sci. 772 (1995).
2. D.M. Pardoll and A.M. Beckerieg, Immunity 3, 165 (1995).
3. W.M. McDonnell and F.K. Askari, N. Engl. J. Med. 334, 42 (1996).
4. J.B. Ulmer et al., Science 259, 1745 (1993).
5. B. Wang et al., Proc. Natl. Acad. Sci. USA 90, 4156 (1993).
6. G.J.M. Cox, T.J. Zamb, L.A. Babiuk, J. Virol. 67, 5664 (1993).
7. E. Raz et al., Proc. Natl. Acad. Sci. USA, 91,9519 (1994).
8. Z.Q. Xiang et al., Virology 199, 132 (1994).
9. J.J. Donnelly et al., J. Infect. Dis. 713, 314 (1996).
10. D.L. Montgomery et al., DNA. Cell. Biol. 12, 777 (1993).
11. J.J. Donnelly et al., Nature Medicine 1, 583 (1995).
12. G.H. Rhodes et al., Dev. Biol. Stand. 82, 229 (1994).
13. H.L. Davis, M.L. Michel, R.G. Whalen, Human Molecular Genetics 2, 1847 (1993).
14. J.B. Ulmer et al., Vaccine 12, 1541 (1994).
15. Z. Xiang and H.C.J. Ertl. Immunity 2, 129 (1995).
16. E.F. Fynan et al, Proc. Natl. Acad. Sci. USA 90, 11478 (1993).
17. E. Manickan, R.J.D. Rouse, Z. Yu, J. Immunol. 155, 259 (1995).
18. M. Sedegah, R. Hedstrom, P. Hobart, S.L. Hoffman, Proc. Natl. Acad. Sci. USA 91, 9866 (1994).
19. M.A. Barry, W.C. Lai, S.A. Johnston, Nature 377, 632 (1995).
20. D. Xu and F.Y. Liew, Vaccine 12, 1534 (1994).
21. D.B. Lowrie, R.E. Tascon, M.J. Colston, Vaccine 12, 1537 (1994).

22. J.W. Moulder, *Microbiol. Rev.* 55, 143 (1991).
23. J. Schachter, *Curr. Top. Microbiol. Immunol.* 138, 109 (1988).
24. S.D. Hillis and J.N. Wasserheit, *N. Engl. J. Med.* 334, 1399 (1996).
25. R.C. Brunham and R.W. Peeling, *Infectious Agents and Disease* 3, 218 (1994).
26. R.P. Morrison, D.S. Manning, H.D. Caldwell, in *Advances in Host Defence Mechanisms*, T.C. Quin, Ed. (Raven Press, New York, 1992), pp 57-84.
27. J.T. Grayston and S.-P. Wang, *Sex. Trans. Dis.* 5, 73 (1978).
28. J.T. Grayston and S.-P. Wang, *J. Infect. Dis.* 132, 87 (1975).
29. H.R. Taylor, J. Whittum-Hudson, J. Schachter, *Invest. Ophthalmol. Vis. Sci.* 29, 1847 (1988).
30. B.E. Batteiger, R.G. Rank, P.M. Bavoil, *J. Gen. Microbiol.* 139, 2965 (1993).
31. M. Campos et al., *Invest. Ophthalmol. Vis. Sci.* 36, 1477 (1995).
32. H. Su, M. Parnell, H.D. Caldwell, *Vaccine* 13, 1023 (1995).
33. T.-W. Tan, A.J. Herring, I.E. Anderson, *Infect. Immun.* 58, 3101 (1990).
34. M. Tuffrey, F. Alexander, W. Conlan, *J. Gen. Microbiol.* 138, 1707 (1992).
35. Y.-X. Zhang, J.G. Fox, Y. Ho, *Mol. Biol. Evol.* 10, 1327 (1993).
36. R.P. Morrison, K. Feilzer, D.B. Tumas, *Infect. Immun.* 63, 4661 (1995).
37. H. Su and H.D. Caldwell, *Infect. Immun.* 63, 3302 (1995).
38. J.U. Igiertseme et al., *Reg. Immunol.* 5, 317 (1993).
39. J.U. Igiertseme and R.G. Rank, *Infect. Immun.* 59, 1346 (1991).
40. D.M. Williams, J. Schachter, J.J. Coalson, *J. Infect. Dis.* 149, 630 (1984).
41. G. Tipples and G. McClarty, *J. Biol. Chem.* 270, 7908 (1995).

42. X. Yang, K.T. HayGlass, R.C. Brunham, J. Immunol., 156, 4338 (1996).
43. Tang et al., Nature 1992, 356: 152-154.
44. Furth et al., Vaccine 1994, 12: 1503-1509.
45. Morrison RP, Manning DS, Caldwell HD. Immunology of *Chlamydia trachomatis* infections: Immunoprotective and immunopathogenetic responses. In: Quin TC. Advances in host defence mechanisms. Sexually transmitted diseases. Vol. 8. New York: Raven Press, 1992: 57-84.